

**ALLELOPATHIC TANNINS FROM GUAVA (*Psidium guajava* L.) LEAF
EXTRACT AGAINST THE RICE WEED, *Echinochloa crus-galli***

JASMIN SALVADOR PAGADUAN



An Undergraduate Thesis Submitted to the Faculty of the Department of Chemistry,
College of Arts and Sciences, Central Luzon State University,
Science City of Munoz, Nueva Ecija, Philippines
in Partial Fulfillment of the requirements
for the degree of

BACHELOR OF SCIENCE IN CHEMISTRY

June 2017

CHE-02-17-009

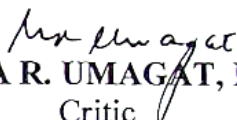
APPROVAL AND ACCEPTANCE

The undergraduate thesis entitled **ALLELOPATHIC TANNINS FROM GUAVA (*Psidium guajava* L.) LEAF EXTRACT AGAINST THE RICE WEED, *Echinochloa crus-galli*** prepared and submitted by **JASMIN S. PAGADUAN** in partial fulfilment of the requirements for the degree of **BACHELOR OF SCIENCE IN CHEMISTRY** is hereby

APPROVED:


ROSALIE R. RAFAEL, Ph.D.
Adviser

6/12/17
Date


MYRNA R. UMAGAT, Ph.D.
Critic

6/13/17
Date



REDEL L. GUTIERREZ, Ph.D.
Department Research Coordinator

6/13/17
Date

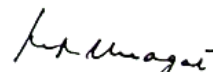

DANILA S. PARAGAS, M.Sc.
Department Chair

6/13/2017
Date

AND ACCEPTED:


ANNA MA. LOURDES S. LATONIO, Ph.D.
College Research Coordinator

6/13/17
Date


MYRNA R. UMAGAT, Ph.D.
Dean

6/13/17
Date

BIOGRAPHICAL SKETCH

PERSONAL INFORMATION

Name : **JASMIN S. PAGADUAN**
Date of birth : September 28, 1995
Place of birth : Talavera, Nueva Ecija

EDUCATIONAL BACKGROUND

Elementary : **DEPED-CLSU ELEM. LAB. SCHOOL**
Science City of Muñoz, Nueva Ecija
March 2008
Secondary : **MUNOZ NATIONAL HIGH SCHOOL**
Science City of Muñoz, Nueva Ecija
March 2012
Tertiary : **CENTRAL LUZON STATE UNIVERSITY**
Science City of Muñoz, Nueva Ecija
Bachelor of Science in Chemistry
June 2017

SCHOLARSHIP AND AWARDS

Dean's list : 1st and 2nd Sem 2012-2013
2nd Sem 2015-2016

EXTRACURRICULAR ACTIVITIES

Lady Master Initiator : CLSU Chemical Society (A.Y. 2015-2016)
Member : Philippine Association of Chemistry Students
(2013-Present)
PRO : Chemistry Student Council (A.Y. 2014-2015)

SEMINARS ATTENDED

Participant : Shimadzu Technology for Analytical
Instrumentation

CAS Little Theater, CLSU
October 8, 2015

Participant : PhilRice Nutrition Month Seminar
PhilRice Central Experiment Station
July 30, 2013

Participant : Chemistry is Fun
Chemistry Symposium Seminar
University Auditorium, CLSU
February 19, 2013

Participant : Breathless: Smoking and Its Impact on One's
Health
University Auditorium, CLSU
September 14, 2012

ACKNOWLEDGEMENT

I give my utmost gratitude to the following people who in one way or another contributed in the completion of this study.

Above all, I am indebted to our Almighty God for the wisdom, strength and well-being that helped me surpass all the trials I have encountered. I thank Him for everything He had done in my life.

To my thesis adviser, Dr. Rosalie R. Rafael who supported and guided me throughout the conduct and writing of this study. Without her thoughtful encouragement and careful supervision, this thesis would never have taken shape. I am blessed to have spent time in her tutelage.

To my critic, Dr. Myrna R. Umagat, for her professional advice for the refinement of this work. I am extremely thankful and indebted to her for sharing expertise and valuable guidance extended to me.

To the faculty members of the Department of Chemistry especially to the Department Research Coordinator, Dr. Redel L. Gutierrez, and Department Chair, Prof. Danila S. Paragas, for their help and continuous encouragement to make this study possible and for teaching and molding me as a future chemist.

To my beloved friends, Hannah, Roxanne, Rochelle, Jemalyn, Justine, Mac especially to my closest college buddies, Cristel and Joahna who have been so supportive and cheering and for the years of friendship that I will constantly cherish.

To my brods and sisses in CLSU Chemical Society, thank you for being a family and for contributing to my personal growth. College is never easy but you make it bearable and memorable.

To my TEAM SEPANX, Andrei and Sarah-P, who have been invaluable on both academic and personal level, for which I am truly grateful. Thank you for teaching me to relish life even under pressure. Thank you for the unforgettable moments.

To my partner, Reupert Ivan, who has been my stress absorber, best support system, and constant taga-hatid-sundo and all. Thank you for never leaving my side and for showing and making me feel the words I should be hearing especially when I was at my lowest. I appreciate everything.

And to my main source of inspiration, my family, especially Mama, Tatay Jun, Kuya Benjo, John, Anniah, Nanay Unday and Tatay Lolong who I would like to express my wholehearted thanks for their generous support (emotionally and financially) they provided me throughout my entire life. Because of their unconditional love and prayers, I was inspired to finish this.

MAY THE ALMIGHTY GOD RICHLY BLESS ALL OF YOU.

JASMIN S. PAGADUAN

TABLE OF CONTENTS

TITLE PAGE	i
APPROVAL AND ACCEPTANCE	ii
BIOGRAPHICAL SKETCH	iii
ACKNOWLEDGEMENT	v
LIST OF FIGURES	ix
LIST OF TABLES	x
LIST OF APPENDICES	xi
LIST OF APPENDIX FIGURES	xii
LIST OF APPENDIX TABLES	xiii
ABSTRACT	1
1 INTRODUCTION	
1.1 Background of the Study	2
1.2 Significance of the Study	3
1.3 Objectives of the Study	4
1.4 Time and Place of the Study	4
1.5 Scope and Limitations of the Study	5
2 REVIEW OF RELATED LITERATURE	
2.1 <i>Psidium guajava</i> L.	6
2.2 Allelopathy	8
2.3 Tannin	10
2.4 <i>Echinochloa crus-galli</i>	11
3 METHODOLOGY	
3.1 Materials, Chemicals, and Reagents	13
3.2 Sample Collection and Preparation	13
3.3 Preparation of the Plant Extract	13
3.4 Tests for tannin of <i>Psidium guajava</i> L. crude extract	14
3.4.1 Test with ferric chloride solution	14
3.4.2 Test with gelatin solution	14
3.5 Extraction of Tannins from <i>Psidium guajava</i> L.	14

3.6 Thin Layer Chromatography Tannins from <i>Psidium guajava</i> L.	15
3.6.1 Preparation of the Developing Chamber	15
3.6.2 Development of the Chromatogram	15
3.6.3 Visualization and Documentation of the Chromatogram	15
3.7 Determination of Total Tannins from <i>Psidium guajava</i> L.	16
3.8 Weedicide Bioassay of Tannins from <i>Psidium guajava</i> L.	16
3.8.1 Test for seed germination of <i>Echinochloa crus-galli</i>	16
3.8.2 Test for seedling growth of <i>Echinochloa crus-galli</i>	17
3.9 Characterization of tannins from <i>Psidium guajava</i> L.	17
3.9.1 UV spectrophotometry of tannins from <i>Psidium guajava</i> L.	17
3.9.2 IR spectrophotometry of tannins from <i>Psidium guajava</i> L.	17
3.10 Statistical Analysis	17
4 RESULTS AND DISCUSSION	
4.1 Extraction of the Bioactive Component of <i>Psidium guajava</i> L.	19
4.2 Tests for Tannin of the Crude Extract and Fractions of <i>Psidium guajava</i> L.	19
4.3 Determination of Total Tannin from <i>Psidium guajava</i> L.	23
4.4 Weedicide bioassay of Tannin from <i>Psidium guajava</i> L.	23
4.5 Characterization of Tannin from <i>Psidium guajava</i> L.	27
4.5.1 UV-Vis Spectrophotometry of Tannin from <i>Psidium guajava</i> L.	27
4.5.2 IR spectrophotometry of Tannin from <i>Psidium guajava</i> L.	27
5 SUMMARY, CONCLUSION AND RECOMMENDATION	
5.1 Summary	30
5.2 Conclusion	31
5.3 Recommendation	32
LITERATURE CITED	33
APPENDICES	39

LIST OF FIGURES

Figure No.	Title	Page
2.1	Images of <i>Psidium guajava</i> L. plant	7
2.2	Chemical structure of (a) gallotannin and (b) gallic acid	11
2.3	Image of <i>Echinochloa crus-galli</i>	12
4.1	Crude extract of <i>Psidium guajava</i> L.	19
4.2	Summary of the positive results of (a) leaf extract of <i>Psidium guajava</i> L. in (b) gelatin test and (c) FeCl ₃ test	20
4.3	Thin layer chromatography of the (a) tannin fraction of <i>Psidium guajava</i> L. leaf extract and (b) standard gallic acid	22
4.4	Inhibitory effect of the tannin concentrations on the seedlings of <i>Echinochloa crus-galli</i>	26
4.5	Absorption spectrum of tannin from <i>Psidium guajava</i> L.	27
4.6	Infrared spectrum of tannin from <i>Psidium guajava</i> L.	28

LIST OF TABLES

Table No.	Title	Page
4.1	Tests for tannins of the fractions of <i>Psidium guajava</i> L.	21
4.2	The effect of different concentrations of the tannin from <i>Psidium guajava</i> L. on the seed germination of <i>Echinochloa crus-galli</i>	23
4.3	Seedling length (mm) of <i>Echinochloa crus-galli</i> subjected to different concentrations of tannin from <i>Psidium guajava</i> L.	24
4.4	Comparison of the infrared positions of tannins from <i>Psidium guajava</i> L. and standard gallic acid	29

LIST OF APPENDICES

Appendix No.	Title	Page
1	Documentation	40
2	Weedicide Bioassay of the Tannin from Guava Leaves	41
3	Data of the Weedicide Bioassay	43
4	Statistical Results	45
5	Computations	48

LIST OF APPENDIX FIGURES

Appendix Figure No.	Title	Page
1.1	<i>Psidium guajava</i> L. crude extract	40
1.2	Preparation of the column	40
1.3	Tannin fraction	40
1.4	Gelatin test for tannin	40
1.5	FeCl ₃ test for tannin	40
2.1	Percent germination of <i>Echinochloa crus-galli</i> seeds (Trial 1)	41
2.2	Percent germination of <i>Echinochloa crus-galli</i> seeds (Trial 2)	41
2.3	Percent germination of <i>Echinochloa crus-galli</i> seeds (Trial 3)	41
2.4	Growth of <i>Echinochloa crus-galli</i> seedlings at different concentrations of tannin (Trial 1)	42
2.5	Growth of <i>Echinochloa crus-galli</i> seedlings at different concentrations of tannin (Trial 2)	42
2.6	Growth of <i>Echinochloa crus-galli</i> seedlings at different concentrations of tannin (Trial 3)	42
4.1	Standard calibration curve of gallic acid	48

LIST OF APPENDIX TABLES

Appendix Table No.	Title	Page
3.1	Mean lengths (mm) of the three trials of the <i>Echinochloa crus-galli</i> seedlings	44
4.1	Analysis of Variance for the initial lengths of <i>Echinochloa crus-galli</i> seedlings	45
4.2	Analysis of Variance for the final lengths of <i>Echinochloa crus-galli</i> seedlings	45
4.3	Post-hoc analysis of final lengths of <i>Echinochloa crus-galli</i> seedlings subjected to different tannin concentrations	46
4.4	Tukey's analysis of final lengths of <i>Echinochloa crus-galli</i> seedlings subjected to different tannin concentration	46
4.5	Analysis of Variance for the difference in the final and initial lengths of <i>Echinochloa crus-galli</i> seedlings	46
4.6	Post-hoc analysis of the difference in the final and initial lengths of <i>Echinochloa crus-galli</i> seedlings subjected to different tannin concentrations	47
4.7	Tukey's analysis of the difference in the final and initial lengths of <i>Echinochloa crus-galli</i> seedlings subjected to different tannin concentrations	47
5.1	Absorption of the tannin from <i>Psidium guajava</i> L.	49

ALLELOPATHIC TANNINS FROM GUAVA (*Psidium guajava* L.) LEAF EXTRACT AGAINST THE RICE WEED, *Echinochloa crus-galli*¹

JASMIN S. PAGADUAN

ABSTRACT

Background: The application of plant allelopathy has been suggested for weed management to minimize the use of synthetic herbicides. This study aimed to extract tannins from *Psidium guajava* L. and determine its allelopathic effect on the seed germination and seedling growth of *Echinochloa crus-galli* rice weed. **Methods:** The dried powdered leaves of *Psidium guajava* L. were extracted using 80% ethanol. The crude extract was further extracted for tannins using 50% acetone. Chemical tests for tannins were done on the leaf extract and fractions of *Psidium guajava* L. and the total tannin content was also obtained. The allelopathic effect of the tannin from *Psidium guajava* L. on the seed germination and seedling growth of *Echinochloa crus-galli* was evaluated. The tannin was characterized using UV-Vis and IR spectrophotometry. **Results:** The total tannin content of the leaf extract was found to be 50.9487 ± 0.00 mg GAE/g dry weight. The percent germination of *Echinochloa crus-galli* was recorded to be $76.67 \pm 4.71\%$ in the control and 0 in all the treatments. The tannin from *Psidium guajava* L. significantly affected the seedling growth of *Echinochloa crus-galli* as indicated by a reduction in the elongation of the seedlings. However, the inhibitory effects of the tannin concentrations were not significantly different from each other but significantly different from the control. The probable tannin present in the leaf extract could be a gallotannin based from the UV-Vis and IR spectra. **Conclusion:** The tannins from *Psidium guajava* L. leaf extract has allelopathic effect against *Echinochloa crus-galli* by inhibiting the seed germination and seedling growth. It has the potential as a weedicide.

Keywords: tannin, *Psidium guajava* L., allelopathy, *Echinochloa crus-galli* rice weed

¹An undergraduate thesis presented to the Faculty of the Department of Chemistry, College of Arts and Sciences, Central Luzon State University, Science City of Muñoz, Nueva Ecija in partial fulfillment of the requirements for the degree of Bachelor of Science in Chemistry. Prepared under the supervision of Professor Dr. Rosalie R. Rafael and Professor Dr. Myrna R. Umagat. June 2017. CHE-02-17-009

LITERATURE CITED

- Abdollahzadeh S.H., Mashouf R.Y., Mortazavi H., Moghaddam M.H., Roozbahani H., and Vahedi M. (2011). Antibacterial and antifungal activities of *Punica granatum* peel extracts against oral pathogens, *Journal of Dentistry*, 8(1), 1–6.
- Abu-Romman S, Shatnawi M, Shibli R.S. (2010). Allelopathic effects of Spurge (*Euphorbia hieroslymitana*) on Wheat (*Triticum durum*). *American-Eurasian J. Agric Environ. Sci.*, 7(3): 298-302.
- Ampong-Nyarko K, De Datta S.K. (1991). A hand book for weed control in rice. International Rice Research institute, 1099 Manila, Philippines.
- Angayarkanni J., Ramkumar K.M., Poornima T. and Priyadarshini U. (2007). Cytotoxic activity of *Amorphophallus paeoniifolius* tuber extracts in vitro. *Am. Eurasian. J. Agric. Environ. Sci.*, 2, 395–398.
- Arowosegbe S., Wintola O.A. and Afolayan A.J. (2012). Phytochemical constituents and allelopathic effect of *Aloe ferox* Mill. root extract on tomato. *Journal of Medicinal Plants Research* Vol. 6(11), pp. 2094-2099.
- Baghestani A., Lemieux C. and Leroux G.D. (1999). Determination of allelochemicals in spring cereal cultivars of different competitiveness, *Weed Sci.*, 47, 498–504.
- Barrett S.C.H. (1983). Crop mimicry in weeds. *Econ Bot.* 37: 255–282.
- Berens, D.G., Farwig, N., Schaab, G. and Böhning-Gaese, K. (2008). Exotic guavas are foci of forest regeneration in Kenyan farmland. *Biotropica*, 40: 104-112.
- Bertin C, Yang X, Weston L.A. (2003). The role of root exudates and allelochemicals in the rhizosphere. *Plant Soil* 256:67–83.
- Badan Pengawasan Obat dan Makanan (2004). Ekstrak Kental Daun Jambu Biji dalam Monografi Ekstrak Tumbuhan Obat Indonesia. Vol 1.
- Brown, R.L., Tang, C. S. and Nishimoto, R. K. (1983). Growth-inhibition from guava roots exudates. *Hortscience*, 18, 316-318.
- Chapla, T. E., & Campos, J. B. (2010). Allelopathic Evidence in Exotic Guava (*Psidium guajava* L.). *Brazilian Archives of Biology and Technology*, 53(6),

1359–1362.

- Chon S.U. and Kim Y.M. (2004). Herbicidal potential and quantification of suspected allelochemicals from four grass crop extracts, *J. Agron. Crop. Sci.*, 190, 145–150..
- Dayan, F. E., Romagni, J. G., Tellez, M. R., Rimando, A. M., Duke, S. O. (1999). Managing weeds with natural products. *Pesticide Outlook*, 5, 185–188.
- Elgailani I.E.H. and Ishak C.Y. (2016). Methods for extraction and characterization of tannins from some acacia species of Sudan. *Pak. J. Anal. Environ.Chem.* Vol. 17(1).
- Einhelling F.A. (1996). Interactions involving allelopathy in cropping systems, *Agronomy Journal*, 88, 886-893.
- Fachinetto J.M., Bagatini M.D., Durigon J., Silva A.C.F. and Tedesco S.B. (2007). Anti-proliferative effect of infusions of *Achyrocline satureioides* on the *Allium cepa* cell cycle, *Rev. Bras. Farmacogn.*, 17, 49–54.
- Farooq M., Siddique K.H.M., Rehman H., Aziz T., Wahid A. and Lee D. (2011). Rice direct seeding experiences and challenges, *Soil Till. Res.*, 111, 87–89.
- Gibson KD, Fischer AJ, Foin TC, Hill JE (2002). Implications of delayed *Echinochloa spp.* germination and duration of competition for integrated weed management in waterseeded rice. *Weed Res.* 42: 351–358.
- Hagerman A.E., Butler L.G. (1978). Protein precipitation method for the quantitative determination of tannins. *J. Agric. Food Chem.*, 26, 809–812.
- Haida KS, Baron A, Haida K.S. (2011). Phenolic compounds and antioxidant activity of two varieties of guava and rue. *Rev Bras Ciênc Saúde* 28: 11-19.
- Harborne (1987). *Metode Fitokimia. Penuntun Cara Modern Menganalisis Tumbuhan.* Padmawinata K, Soediro E, Penerjemah; Bandung: ITB Pr. Terjemahan dari: *Phytochemical methods.*
- Hegab MM, Khodary SEA, Hammouda O, Ghareib H.R. (2008). Autotoxicity of chard and its allelopathic potentiality on germination and some metabolic activities associated with growth of wheat seedlings. *Afr. J. Biotechnol.* 7(7): 884-892
- Holm L.G., Pancho J.V., Herberger JP, Plucknett D.L. (1979). *A geographical atlas of world weeds.* John Wiley and Sons, New York.

- Isaza, J.H., Ito, H., Yoshida, T. (2004). Oligomeric hydrolysable tannins from *Monochaetum multiflorum*. *Journal of Phytochemistry* 65, 3:359-364.
- Jabeen, N. and M. Ahmed. (2009). Possible allelopathic effects of three different weeds on germination and growth of maize (*zea mays*) cultivars. *Pak. J. Bot.* 41(4): 1677-1683.
- Jamil M., Cheema Z.A., Mushtaq M.N., Farooq M. and Cheema M.A. (2009). Alternative control of wild oat and canary grass in wheat fields by allelopathic plant water extracts, *Agron. Sustain. Dev.*, 29: 475–482.
- Kawawa, R.C.A., Obiri, J.F. ., Muyekho F. N ., Omayio, D.O., Agevi, H., Mwaura, A., Obiet, L., Kimutai, D.K., and Sifuna A.W. (2016). Allelopathic potential of invasive *Psidium guajava* L. against selected native tree species in Kakamega Tropical Forest, Western Kenya. *Journal of Pharmacy and Biological Sciences*, 11(5): 80-86.
- Kebriaee-zadeh A. (2003). Overview of national drug policy of Iran. *Iranian J. Pharm. Res.* 2:1–2.
- Kinghorn, L. Pan, J.N. Fletcher, H. Chai (2011). The relevance of higher plants in lead compound discovery programs *J. Nat. Prod.*, 74: pp. 1539–1555.
- Kruse M, Strandberg M, Strandberg B. (2000). Ecological effects of allelopathic plants. A review, Department of Terrestrial Ecology, Silkeborg, Denmark, Rep. p. 315.
- Liu Y, Dao Z, Liu Y, Long C. (2009). Medicinal plants used by the Tibetan in Shangri-la, Yunnan, China. *Ethnobiol. Ethnomed.*;5.
- Lovett J., Ryuntyu M. (1992). Allelopathy: broadening the context: in S. J. H. Rizvi and V. Rizvi (eds.). *Allelopathy, Basic and Applied Aspects*. Chapman & Hall, London., pp. 11-19.
- Machado S. (2007). Allelopathic potential of various plant species on Downy Brome: Implications for weed control in wheat production. *Agron . J .* 99(1): 127-132.
- Meagher, L.P., Lane, G., Sivakumaran, S., Tavendale, M.H., Fraser, K.J. (2004). Characterization of condensed tannins from Lotus species by thiolytic degradation and electrospray mass spectrometry. *Journal of Animal Feed Science and Technology* 117, 1 : 151-163.
- Morton, J.F. (1987). Guava (*Psidium guajava* L.). In: *Fruits of warm climates*, Miami, FL, 356-363.

- Muscolo A., Panuccio M.R., Sidari M. (2001). The effect of phenols on respiratory enzymes in seed germination respiratory enzyme activities during germination of Pinus.
- Nam S., Smith D.M. and Dou Q.P. (2001). Tannic acid potently inhibits tumor cell proteasome activity, increases p27 and Bax expression, and induces G1 arrest and apoptosis, *Cancer Epidemiol. Biomarkers Prev.*, 10(10): 3-1088.
- Ohira T, Yatagai M (1994). Allelopathic compounds produced by forest plant. II. The relationships between the inhibition effects on plant growth and killing activities of brine shrimp on phenolic compounds. *Mok. Gak.*, 40: 541-548.
- Olofsdotter, M. (2001). Rice – A step toward use of Allelopathy. *Agronomy Journal*. 93: 3–8.
- Pantoja-Castroa M., and González-Rodríguez H., (2011). Study by infrared spectroscopy and thermogravimetric analysis of Tannins and Tannic Acid Latinoamer. *Quím.* 39/3.
- Patterson D.T. (1985). Comparative ecophysiology of weeds and crops. In: Duke SO (ed.), *Weed Physiology, Vol. I: Reproduction and Ecophysiology*, Boca Raton, FL: CRC Press, pp 101–130.
- Price M.L, Van Scoyoc S., Butler L.G. (1978). A critical evaluation of the vanillic reaction as an assay for tannin in sorghum grain. *J. Agric. Food Chem.*, 8, 26, 1214–1218.
- Putnam A.R. (1985). Weed allelopathy. In S.O. Duke, (ed.) *Weed Physiology, Volume I: Reproduction and Ecophysiology*. Boca Raton, FL: CRC Press: 131-155.
- Rao C.S. (2004). Evaluation of Anti-Bacterial Activity with Tannin Fraction from *Psidium guajava* leaves and barks, Volume 3(4): ISSN:2321-6182.
- Rice E.L. (1984). *Allelopathy*. London: Academic Press.
- Rita, Y. (2006). Kandungan Tanin dan Potensi Anti Streptococcus Mutans Daun The Varietas Assamica pada Berbagai Tahap Pengolahan. Skripsi. Institut Pertanian Bogor, Bogor. Juni.
- Ryu NH, Park KR, Kim SM, Yun HM, Nam D, et al. (2012) A Hexane Fraction of Guava Leaves (*Psidium guajava L.*) Induces Anticancer Activity by Suppressing AKT/Mammalian Target of Rapamycin/Ribosomal p70 S6 Kinase in Human Prostate Cancer Cells. *J Med Food* 15: 231-241.

- Samuelson G. (1999). *Drugs of Natural Origin: A Textbook of Pharmacognosy*. Taylor & Francis Ltd., ISBN 9789186274818.
- Shutava, T., Prouty, M., Kommireddy, D., & Lvov, Y. (2005). pH responsive decomposable layer-by-layer nanofilms and capsules on the basis of tannic acid[^]. *Macromolecules*, 38: 2850–2858.
- Silverstein, R.M., Bassler, G.C., Morrill, T.C. (1981). *Spectrometric Identification of Organic Compounds*. 7th ed., John Wiley & Sons Inc., USA pp: 95-98.
- Socrates, G. (2004). *Infrared and Raman Characteristics Group Frequencies, Tables and Chart*. 3a. ed., John Wiley & Sons, Inc., USA pp: 125-142.
- Stuart, B. H. (2005). *Experimental Methods in Infrared Spectroscopy: Fundamentals and Applications*, John Wiley & Sons, Ltd, Chichester, UK pp: 76-77.
- Tamilarashi, U. Subasini, S. Kavimani and B. Jaykar. (2000). Phytochemical and pharmacological evaluation of *Ampelocissus latifolia*, *Anc. Sci. of Life*, 20(1), 14-18.
- Tambe V.D., and Bhambar R.S. (2014). Estimation of total phenol, tannin, alkaloid, and flavonoid in *Hibiscus tillaceus L.* wood extracts. *Journal of Pharmacognosy and Phytochemistry Vol 2(4)*.
- Teixeira Ro, Camparoto Ml, Mantovani Ms And Vicentini Vep. (2003). Assessment of two medicinal plants, *Psidium guajava L.* and *Achillea millefolium L.*, in vitro and in vivo assays. *Genet Mol Biol* 26: 551-555.
- Vidotto F., Tesio F., Tabacchi M., Ferrero A. (2007). Herbicide sensitivity of *Echinochloa spp.* accessions in Italian rice fields. *Crop Prot.* 26: 285–293.
- Vyvyan J.R. (2002). Allelochemicals as leads for new herbicides and agrochemicals, *Tetrahedron*, 58(9), 1631-1646.
- Winkel-Shirley B. (2002). Biosynthesis of flavonoids and effects of stress, *Curr. Opin. Plant Biol.*, 5, 218-223.
- Yugan Tar Kumar, and A. N. Sahu B.Pharm. (2013). *Pharmacognosy-II Course Code: Ph-3102 Department Of Pharmaceutics Iit (Bhu), Varanasi . Part-3rd Roll No. 11121.*
- Yusof S.G. (2003). *University Putra Malaysia, Selangor, Malaysia. Elsevier Science Ltd 2985- 2991.*